**Summary of Discussion on "Road Map" of Rice Functional Genomics
1 to 4 p.m., February 24, 2006
Woodlands Waterway Marriott, Woodlands, TX**

Background

The USDA-CSREES National Research Initiative (NRI) program organized an overview meeting on February 24, 2006, for all USDA-NRI funded projects. The event, scheduled in conjunction with the Rice Coordinated Agricultural Project (RiceCAP) meeting, promoted interaction and linkages among researchers involved in NRI and the RiceCAP programs. The NRI competitive grant program has supported 13 projects related to rice functional genomics since 2003. The meeting's morning session was devoted to presentation of individual projects. The afternoon session centered on the following objectives:

- Enable more interactions among principal investigators (PIs) of projects involved in rice functional genomics
- Generate discussion on the "road map" of rice functional genomics in the context of national and international collaboration
- Gather ideas as inputs for guiding future direction of rice functional genomics activities.

Participants

Ed Kaleikau, USDA-CSREES
Duane Alphs, USDA-CSREES
Jiming Jiang, University of Wisconsin-Madison
Benildo de los Reyes, University of Maine
Venkatasen Sundaresan, University of California-Davis
Blake Meyers, University of Delaware
Zhaohua Peng, Mississippi State University
Dick McCombie, Cold Spring Harbor Laboratory
Luca Comai, University of Washington
Robin Buell, The Institute for Genomic Research
Kevin Child, The Institute for Genomic Research
Naohiro Kato, Louisiana State University
Ning Jiang, Michigan State University
David Galbraith, University of Arizona

Bill Crosby, University of Windsor
Hei Leung, International Rice Research Institute
Ron Phillips, University of Minnesota
Kay Simmons, USDA-Agricultural Research Service (ARS)

The session began with a presentation on the International Rice Functional Genomics Consortium (IRFGC), which provided a summary of the background, objectives, and organization of the consortium. The basic premise of a consortium approach is that it can harness the collective research efforts to achieve larger goals. Collaboration in functional genomics is a necessity because of the diversity of genetic resources, expertise, and agronomic environments needed for understanding gene function. A consortium approach can benefit from the synergy of collaboration and avoid redundancy. While the philosophy of a consortium is well accepted, there is a need to: a) set realistic and achievable targets, b) identify systematic approaches, and c) develop a road map that is broadly accepted by the research community. With its open grant programs, U.S. researchers have (and will continue to) played a key role in shaping the agenda.

The discussion was organized around the leading questions posed by USDA (Ed Kaleikau):
- What are the knowledge gaps and research, education, and extension opportunities in rice functional genomics?
- How can we move the field of rice functional genomics forward to have the greatest impact? What research is needed to provide the best return on investment to the U.S. taxpayer?
- What would a broad road map of this research look like?

Hei Leung served as moderator and Blake Meyers recorded the key points from the discussion. In the discussion points outlined below, some of the responses are posed as questions, with the goal of stimulating further thought and discussion about the U.S. rice research focus.

Knowledge Gaps/Questions
- If a long-term goal of rice functional genomics is to understand the function of all agriculturally important genes, we need to define what constitutes a meaningful description of gene function. Also the "definition of genes" is expanding as we learn more about noncoding RNAs, alternative splice products etc. It is therefore important to incorporate the expanding meaning of a basic unit of a gene for the purpose of analysis.

- What can we learn from Arabidopsis 2010 Project?

The current goal is a very basic understanding of genes – where are they expressed and where are the gene products localized. The goal of understanding the function of every gene is perhaps a bit naïve. For rice, an alternative, and perhaps more realistic, goal is to focus on pathways, networks, and systems and emphasize those traits or biological aspects which are unique to rice.

We could use a multi-tiered approach to focus on genes of agricultural importance, those which are important to rice, and avoid duplication with Arabidopsis. This prioritization would be incomplete but might capture the most genes and reduce the overall investment, while getting the most impact. It seems that regardless of the "depth" of functional description, a systematic approach is important to achieve the objectives within a certain time frame.

If we are to maximize the returns (scientific and economic) from the investment in rice genomics, there is a need to continue to advance rice as a better and desirable crop and

genetic model for young scientists and plant biologists. This can be done in part by developing a framework of resources that will facilitate research. The tools that we have now are good, but may not be adequate for the long term. Continued investment in resources/tools is important to maintain the utility of rice as a model. Some key elements include:

- Recognizing that many knockouts are non-penetrant and that there are many cases of genetic redundancy; knockout lines alone are perhaps not sufficient. Thus, in terms of genetic resources, we need to develop enough genetic diversity for a variety of investigations. This implies tapping into the rich pool of diversity in rice and/or devising novel ways to induce variation.
- Large scale genomic annotation/transcript analyses are important for defining the transcribed regions and providing basic parameters about expression; continued development of these resources is important but should be justified based on the amount of new data obtained with each new project.
- Find a balance among these activities and investments for just a single genotype versus multiple genotypes of indica/japonica rice (to make use of the diversity).
- Need a continual assessment of whether the research community has achieved a saturation point in terms of knowledge that would suggest further investment might not be beneficial. As new questions come up, the cost/benefit analysis of certain resources/investment should be revisited.
- Find the most efficient means to add value and biological meaning to the many rice genes and alleles discovered from sequencing. This is another depth of analysis; systematic approaches are required to span this gap in knowledge.

Research Opportunities
Mutant Stocks
1. Phenotypic valuation of knockout lines. Rich source of stocks exists, and these could be measured for specific traits. The utility of these lines can be enhanced by classifying them according to phenotype or trait ontology.
2. The genetic depths have not yet been fully explored, which is an issue of understanding rice genetic diversity. Identifying groups of mutants or germplasm with similar traits may be beneficial to both molecular biologists and breeders.
3. High-throughput phenotyping is a rate limiting step, particularly for more complex traits. This deserves a higher level of investment if the community can agree on phenotypes that are most valuable to identify and coordinate their efforts in evaluating the end products. We may challenge breeders/physiologists to develop ways to evaluate thousands of lines to identify those of interest, which can serve as specialized genetic stocks for further study. Examples include:
   a) Analysis of specific domestication traits.
   b) Nutritional traits, e.g., iron metabolism
   c) Drought-tolerance traits
4. Standardization of definitions for phenotyping. There are on-going efforts through the gene/trait/phenotype ontology consortia but it is not clear where rice stands relative to other crops/plants.

Gene Expression
1. A gene expression atlas that is organized on a set of tissues/treatments with very broad coverage will be highly useful to scientists and students, regardless of the technology platform. It is noted that efforts to produce these resources may already exist, including the Deng/Nelson NSF project, TIGR's gene expression database, or the MPSS dataset. Access to some of these data, however, may be the limiting factor at the moment. Although these

resources may be in the pipeline, they may require more momentum and publicity to broaden access to these resources.

2. Consider developing resources of cell stocks/lines, RNA with standards. In other words, dissect the plant into more objects as experimental materials for investigation and distribution.

3. Consider a full-length cDNA clone collection and correct annotation for every expressed gene in rice. This could be held at a stock center for overexpression, protein interaction studies. Having a large and complete library would facilitate many projects as an enabling resource because it would save work by individual researchers. This may improve the status of rice as a model cereal.

4. The pros and cons of a focused approach to understand in-depth a single variety such as Nipponbare. There are different opinions regarding the benefits of understanding Nipponbare in-depth versus the alternative of understanding broad phenotypic diversity using multiple genotypes. One reason is that Nipponbare may not be the preferred genotype for phenotyping a large number of agronomic traits. More opinions and inputs from researchers working in different parts of the world are needed.

Issue of a Rice Stock Center

1. Propagation of transgenic seed stocks and molecular resources (clones, libraries, DNA, etc). Given the large investment already made in the production of the resources, establishing a center to store and distribute such resources is well justified. In Arabidopsis, the Ohio State stock center is used extensively. For rice, a similar system would be most helpful to the community.

2. For rice, the issue may be the bulkiness of rice seed compared to Arabidopsis. It is important that rice resources be freely available, as this will lead to buy-in by smaller groups who will follow the example. A stock center should be willing and able to multiply seed for re-distribution, rather than just a mailing service.

3. As a community, we could institute a rule that any published papers that describe lines/materials should be submitted to a public stock center (e.g., Dale Bumpers or other international repositories).

4. Establishing a rice stock center was recognized as a need at least two years ago. The community anticipated a strong effort by the Dale Bumpers National Rice Research Center to solicit lines/materials to start to build the resources and prioritize acquisitions. Also, a stock center should advertise the materials that it can provide.

5. Kay Simmons and Ed Kaleikau elaborated on the current status of the stock center and related activities:
    *   Interagency Working Group on Plant Genomes (USDA, NSF, DOE, NASA, NIH, USAID) has an agreement that USDA-ARS is responsible for the rice seed genetic stocks. In 2003, USDA-ARS wrote a letter of intent stating that ARS is committed to long-term storage and distribution of rice seed stock resources generated from NSF Plant Genome Program and NRI Plant Genome awards. ARS intends to distribute the seed stocks freely to the scientific community and will not accept accessions accompanied by Material Transfer Agreements. ARS will accept accessions that contain transgenes, but will require that any accession of a "regulated" item be accompanied by required permits from APHIS or the appropriate regulatory agency. In 2004 ARS established the Genetic Stocks – Oryza (GSOR) Collection and the website is: http://www.ars.usda.gov/Main/docs.htm?docid=8318
    *   At this meeting, Anna McClung, Director of the Dale Bumpers National Rice Research Center announced that ARS received funding in Fiscal Year 2006 to support a new Rice Geneticist position for the Rice Seed Genetics Stock Center. This new position will be

advertised soon and will be similar to that of Marty Sachs at the USDA-ARS Maize Genetic Stock Center at Illinois. The new position will:
   a. Curate and distribute existing rice seed genetic stocks
   b. Actively acquire new genetic stocks (germplasm, mutants, mapping populations, wild species)
   c. Develop new genetic stocks and experimental populations to enhance gene discovery
   d. Form partnerships with national and international scientists to expand the development of specialized genetic materials
- TILLING resources as an example to build the contents of the Stock Center
   a. NRI funded project on producing TILLING populations of indica and japonica rice. IRRI will deposit the indica IR64 population (~8,000 M4 lines) within 2006. The Nipponbare population will come from UC-Davis (Luca Comai and Tom Tai).
   b. It is expected that genetic stocks and mutants developed from NRI projects (e.g., Sundaresan's mutant project) will be deposited at the DB Center either during or after the funding period.

Natural Variation
1. This is an area of rice biology in which we know relatively little. The community would like to have the tools necessary to carry out the analyses on broader questions of genetic variation and functional diversity. The Rice SNP project coordinated by IRFGC is working in the right direction.
2. It appears that more genomic sequence information from diverse rice germplasm is needed. To make use of the HapMap-like data derived from the 21 lines of the Rice SNP project, the community should consider how to prioritize, classify, access and work with additional lines (perhaps up to a thousand more) in the future.
3. Beyond the HapMap/Perlegen data, the community may consider characterization of major structural variants, and of diversity created by insertions/deletions.
4. Considerable genetic diversity is available at IRRI, which can provide most of these resources, However, for US researchers, the question remains which lines should be sequenced and where investment should be made. We should consider the priority of cultivars and wild relatives that should be held in US stock centers.
5. Considering the impact of recombinant inbred lines in Arabidopsis research, it would be desirable to maintain and/or create a set of standard and immortalized recombinant inbred populations. Such resources will help a large number of projects. RiceCAP RILs will be available. These, together with other available RIL populations, would represent permanent stocks with multiple utility for the long term. The US community together with international partners should discuss what lines and populations should be focused on, and how phenotypic data can be incorporated into this selection process.

Data Integration
1. Interoperability of disparate data sets. Integration of data may soon become the rate limiting step.

Additional ideas
1. Ways to transfer more than a single gene. Possibility to capitalize on advances in engineering artificial chromosomes.
2. Other uses for rice – opportunities for energy production? May be C4 grasses are better candidates.
3. Development of RNAi constructs/clone libraries as a common resource.
4. Stochasticity in gene expression (perhaps rice isn't the best system in which to study this).

Education Opportunities

1. Bridging the gap between molecular biologists and breeders is important and necessary – something that has not been done adequately yet. This is both an education and extension opportunity.
2. We could develop rice-based materials and information (seeds, online materials) for high school or other students. One suggestion is to develop a "fast-rice" plant along the lines of the Arabidopsis/Brassica fast cycling strain that is widely used in plant research and teaching biology. A genotype with a short generation time (from seed-to-seed) would show the students the complete life cycle.

Extension Opportunities

1. There is a need to perform better at delivering materials to breeders as they are directly connected to the growers. It is important to get feedback from breeders. This communication must be strengthened.
2. US breeders like the integrity of pure lines and may be reluctant to bring in diversity. Therefore, marker assisted selection or other technologies are needed to facilitate more precise transfer of traits from diverse lines into the elite inbred lines.
3. Breeders may be reluctant to make distant-crosses with their highly-tuned elite lines because of the amount of work to integrate the new genes and at the same time maintain elite qualities and eliminate linkage drag. Low cost, high-throughput, whole-genome methods would be attractive to breeders.
4. Workshops could be used to improve these interactions (perhaps in warm places held in January).

Synergistic Opportunities

1. Increase international collaborations to improve synergy and to reduce redundant efforts. Accessing data/resources at foreign institutions by improved communication/sharing.

Road Map

Out of the free-flow of ideas and discussion, several major themes emerged from the brainstorming session:

1. Adding more tools and resources would continue to improve rice as a model.
2. There is a fundamental gap between molecular and phenotypic information. How to bridge this gap and how to prioritize to make the best use of limited resources should be a key consideration in designing future activities.
3. A centralized stock center for molecular and genetic resources would facilitate research which in turn accelerate uptake in practical applications.
4. Improve ways to publicize available rice resources and make them widely accessible
5. Emphasize rice-specific traits/phenotypes/opportunities to maximize the return from investments.